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Virology 320 (2004) 63–74

VIROLOGY

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The role of inducer cells in mediating in vitro suppression of feline immunodeficiency virus replication

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Received 17 January 2003; returned to author for revision 23 October 2003; accepted 3 November 2003

Abstract

$CD8^+$ T-cell-mediated suppression of feline immunodeficiency virus (FIV) replication has been described by several groups, although the mechanisms of activation and conditions for viral suppression vary with the methodologies. We have previously reported that $CD8^+$ T-cell-mediated suppression of FIV replication required inducer cell stimulation of the effector cells. The focus of the present study was to examine the essential role of inducer cells required for the induction of this soluble anti-FIV activity. Both FIV-PPR-infected T cells and feline skin fibroblasts (FSF) infected with an alphavirus vector expressing FIV capsid or the irrelevant antigen lacZ, stimulated autologous or heterologous effector cells to produce supernatants that suppressed FIV replication. Thus, induction of this suppression of FIV replication did not strictly require autologous inducer cells and did not require the presence of FIV antigen. Anti-viral activity correlated with the presence of $CD8^+$ T cells. Suppression was maximal when the inducer cells and the effector cells were in contact with each other, because separation of the inducer and effector cells by a 0.45-μm membrane reduced FIV suppression by approximately 50%. These findings emphasize the importance for membrane antigen interactions and cytokines in the optimal induction of effector cell synthesis of the soluble anti-FIV activity.

Keywords: FIV; Inducer cells; Feline skin fibroblasts (FSF); Inducer cell effector cell contact; $CD8^+$ T lymphocytes

Introduction

Feline immunodeficiency virus (FIV) is a T-lymphotropic lentivirus in the family Retroviridae (Pedersen et al., 1987). Natural or experimental FIV infection of cats results in a transmissible AIDS-like disease, similar to that observed with human immunodeficiency virus (HIV) infection in humans (Pedersen et al., 1989; Yamamoto et al., 1988).

$CD8^+$ T cells are a critical component of cellular immune protection against HIV infection. $CD8^+$ T lymphocytes control HIV, SIV and FIV infection through both cytotoxic (CTL) and non-cytotoxic mechanisms (Appay et al., 2000; Barker, 1999; Barouch and Letvin, 2001; Blackbourn et al., 1994; Brinchmann et al., 1990; Charaf et al., 1993; Choi et al., 2000; Flynn et al., 2002; Furci et al., 2002; Geiben-Lynn et al., 2001; Hohdatsu et al., 2000; Jeng et al., 1996; Kannagi et al., 1988; Le Borgne et al., 2000; Mackewicz et al., 1994; Pollack et al., 1997; Powell et al., 1990; Song et al., 1992; Walker, 1993; Walker et al., 1986; Yang et al., 1997). Non-cytotoxic responses of $CD8^+$ T lymphocytes, a type of innate immunity, may be more important than the cytotoxic responses in controlling certain viral infections (Levy, 2001). This $CD8^+$ non-cytotoxic antiviral response is thought to be mediated, at least in part, by an undefined secreted $CD8^+$ antiviral factor (CAF) (Clerici et al., 1996; Levy, 2001; Levy et al., 1996). Such non-cytotoxic activity is not HIV-specific nor species-specific, is not restricted by HLA class I or class II molecules and is identified immediately after acute HIV infection (Levy, 2001; Mackewicz et al., 1998; Walker et al., 1991a). The levels of $CD8^+$ anti-HIV responses have been correlated with CD4 counts and the absence of infection in individuals repeatedly exposed to HIV and in HIV-infected, long-term survivors

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doi:10.1016/j.virol.2003.11.016

(Blackbourn et al., 1996; Furci et al., 2002; Gomez et al., 1994; Landay et al., 1993; Levy, 2001; Mackewicz et al., 1991; Stranford et al., 1999). In contrast, Copeland et al. (1997) did not find any correlation between the extent of $CD8^+$ T-cell-mediated suppression and clinical stage of infection or $CD4^+$ T-cell counts in HIV-1-infected individuals.

$CD8^+$ T-cell-mediated suppression of FIV replication has been described by several groups, although the mechanisms of activation and conditions for viral suppression vary with the methodologies (Bucci et al., 1998a; Choi et al., 2000; Crawford et al., 2001; Flynn et al., 1999, 2002; Hohdatsu et al., 1998, 2000, 2002; Jeng et al., 1996). In our studies, reproducible production of non-cytotoxic anti-FIV activity from $CD8^+$ T lymphocytes, from cats in the asymptomatic stage of FIV infection, has been maximally achieved in vitro with effector cells stimulated by FIV-PPR strain-infected inducer T cells rather than mitogen (Choi et al., 2000).

Whereas a number of studies have attempted to characterize the $CD8^+$ T cells responsible for generating lentivirus suppression (Bucci et al., 1998b; Flynn et al., 2002; Gebhard et al., 1999; Landay et al., 1993; Toso et al., 1995; Zanussi et al., 1996) or have focused on characterizing the $CD8^+$ antiviral activity (Geiben-Lynn et al., 2001; Mosoian et al., 2000), there is little known about the physiologic stimulus that activates the effector $CD8^+$ T cells to generate suppressing activity. Previous reports suggested that HIV infection or a potential exposure to the virus was needed for the CAF activity (Levy et al., 1998; Walker et al., 1991b). However, the fact that the $CD8^+$ T-cell antiviral response has been observed in asymptomatic HIV-infected individuals (Blackbourn et al., 1996; Brinchmann et al., 1990; Hsueh et al., 1994; Mackewicz et al., 1991; Toso et al., 1995), HIV exposed but uninfected individuals (Furci et al., 2002; Levy et al., 1998; Stranford et al., 1999) and healthy HIV-naïve individuals (Kootstra et al., 1997; Rosok et al., 1997), as well as in FIV-infected and -uninfected cats (Choi et al., 2000), suggests that mechanisms involved for the induction of this antiviral response are not virus-specific.

Because the $CD8^+$ antiviral activity seems to be non-specific with respect to HIV or FIV, the elicited immune response would seem to be of an innate nature. Both soluble and cellular components contribute to innate immunity (Levy, 2001), resulting in a general response of the immune system to an infection without specificity for a target antigen. Identifying the conditions leading to the induction of this antiviral activity is essential in exploiting this innate control of lentiviral infection. Here we report for the first time, that feline skin fibroblasts (FSF) could be used as inducer cells to mediate the soluble anti-FIV activity and the importance of inducer cell–effector cell contact in the generation of optimal suppression of FIV replication in vitro.

Results

Production of the soluble anti-FIV activity requires stimulation by inducer cells which do not have to be MHC-restricted

We have previously reported that the soluble anti-FIV activity was secreted from PBMC of both FIV-infected and -uninfected cats after stimulation with autologous FIV-infected inducer T cells, in contrast to stimulation with concanavalin A (Con A) (Choi et al., 2000). Supernatants from the PBMC of two uninfected cats OAE5 and OLQ4 and six FIV-infected cats AUO2, AZV2, OLM6, AUO3, OLQ5 and AWF1 induced virus expression rather than suppressing virus replication, when FIV-infected target cells were treated with cell culture supernatants of effector cells not stimulated with infected inducer T cells (Table 1). This confirmed that production of the soluble anti-FIV activity from the effector cells of these animals requires stimulation by exogenously added inducer cells.

It was further determined as to whether inducer and effector cells required MHC compatibility to stimulate the anti-FIV activity. Therefore, inducer T cells were obtained from autologous or heterologous donors as compared to the source of effector cells. Cell culture supernatants collected on day 7, from PBMC stimulated by irradiated MHC-matched autologous or irradiated MHC-mismatched heter-

Table 1
Requirement of FIV-infected inducer T cells for stimulation of the effector cells for the production of the soluble anti-FIV activity

Cats	Viral antigen expression in supernatants from target cells that received supernatants from effector cells which were not stimulated by inducer T cells[a]	
	Capsid optical density (OD)[b]	% Virus expression[c]
FIV (−)		
OAE5	2.18	116.6
OLQ4	2.41	128.9
FIV (+)		
AUO2	2.77	148.1
AUO3	1.74	93.0
AZV2	2.32	124.1
OLQ5	1.8	96.3
OLM6	2.57	137.4
AWF1	2.35	125.7
Control[d]	1.87	100.0

[a] Cell culture supernatants were collected from effector cells (not stimulated by the FIV-infected inducer T cells) after 7 days, ultracentrifuged and added at a medium to supernatant ratio of 1:1 to FIV-PPR-infected target cells every 3 days.
[b] FIV capsid antigen was measured by an FIV capsid antigen detection enzyme-linked immunosorbent assay (ELISA) as mentioned in the Materials and methods. The standard deviations ranged from 0.1 to 0.23 for these values.
[c] Relative to virus expression of control cells.
[d] These control cells were infected with FIV-PPR and cultured in complete medium without effector cell supernatant.

ologous FIV-PPR-infected inducer T cells demonstrated FIV suppressive activities in all cats examined, four FIV-infected (AUO2, AUO3, AZV2 and OLQ5) and two FIV-uninfected cats (AUS3 and OAE5) (Table 2). Supernatants collected on day 7, from PBMC of cats OAE5, AUO2, AZV2 and OLQ5 obtained following stimulation by MHC-matched inducer T cells demonstrated somewhat stronger FIV suppression than supernatants obtained after stimulation by MHC-mismatched inducer T cells (Table 2). In contrast, supernatants collected on day 7, from PBMC of cats AUS3 (FIV-uninfected) and AUO3 (FIV-infected) stimulated by MHC-mismatched inducer T cells demonstrated even more potent suppression of FIV replication, than supernatants from PBMC stimulated by MHC-matched inducer T cells (Table 2). Therefore, the induction of the anti-FIV activity from feline PBMC was not MHC-restricted.

Use of FSF as inducer cells

The studies, thus far, have depended on FIV-infected T cells for efficient induction of the anti-FIV activity. To determine if other types of cells could also be used as inducer cells, SV40-transformed FSF were infected with Semliki Forest virus (SFV) vector recombinant particles expressing FIV capsid. These cells replaced T cells as inducer cells. Supernatants collected on day 7, from cells of the cats whose PBMC were stimulated by autologous irradiated FSF cells expressing FIV capsid, strongly suppressed FIV replication (Table 3). Suppression observed using FSF as inducer cells was quantitatively equivalent to that observed using inducer T cells. Supernatants collected on day 7 from PBMC cultured with autologous irradiated FSF not expressing FIV capsid did not demonstrate detectable FIV-suppressive activity, with the exception of supernatants from PBMC of the two FIV-infected cats AUO3 and AZV2, which suppressed virus replication very weakly (as determined by the 65% viral expression limit). These results indicated that FSF expressing a virus antigen could be used as inducer cells.

$CD8^+$ T lymphocytes are responsible for the suppression of FIV replication when FSF are used as inducer cells

$CD8^+$ T lymphocytes were enriched by negative selection to ensure that the $CD8^+$ T-lymphocyte population

Table 2
Effect of MHC-matched and MHC-mismatched inducer T cell stimulation on the production of the soluble anti-FIV activity

Cats	Viral antigen expression in supernatants from target cells that received supernatants from effector cells which were stimulated by			
	Irradiated MHC-matched Inducer T cells[a]		Irradiated MHC-mismatched Inducer T cells[b]	
	Capsid OD[c]	% Virus expression[d]	Capsid OD	% Virus expression
FIV (−)				
AUS3	1.22 ± 0.31*	34.1	0.73 ± 0.06*	20.4
OAE5	0.77 ± 0.04*	21.5	1.46 ± 0.04*	40.8
FIV (+)				
AUO2	0.47 ± 0.04*	13.1	0.85 ± 0.06*	23.7
AUO3	0.42 ± 0.05*	11.7	0.35 ± 0.01*	9.8
AZV2	1.70 ± 0.26*	47.5	1.77 ± 0.08*	49.4
OLQ5	1.06 ± 0.07*	29.6	1.27 ± 0.05*	35.5
Control[e]	3.58 ± 0.09	100.0	3.58 ± 0.09	100.0

[a] Effector cells were stimulated with irradiated MHC-matched FIV-infected inducer T cells for 7 days. The supernatants collected from the stimulated cells on the seventh day were ultracentrifuged and added at a medium to supernatant ratio of 1:1 to FIV-PPR-infected target cells every 3 days.
[b] Effector cells were stimulated with irradiated MHC-mismatched FIV-infected inducer T cells for 7 days. The supernatants collected from the stimulated cells on the seventh day were ultracentrifuged and added at a medium to supernatant ratio of 1:1 to FIV-PPR-infected target cells every 3 days.
[c] FIV capsid antigen was measured by an FIV capsid antigen detection ELISA as mentioned in the Materials and methods. Data represent the mean ± SE for the experiments.
[d] Relative to virus expression of control cells.
[e] These control cells were infected with FIV-PPR and cultured in complete medium without effector cell supernatant.
* Statistically significant at a P value of <0.05.

Table 3
Induction of the soluble anti-FIV activity by effector cells stimulated by FSF expressing FIV capsid

Cats	Viral antigen expression in supernatants from target cells that received supernatants from effector cells which were stimulated by autologous			
	FSF cells expressing FIV capsid[a]		FSF cells not expressing FIV capsid[b]	
	Capsid OD[c]	% Virus expression[d]	Capsid OD	% Virus expression
FIV (+)				
AUO2	0.08 ± 0.03*	4.1	2.88 ± 0.49	148.5
AUO3	0.05 ± 0.00*	2.6	1.16 ± 0.23*	59.8
AWF1	0.13 ± 0.02*	6.7	2.75 ± 0.70	141.8
AZV2	0.24 ± 0.06*	12.4	1.19 ± 0.56*	61.3
OLQ5	0.40 ± 0.13*	20.6	2.68 ± 0.98	138.1
FIV (−)				
OAE5	0.34 ± 0.07*	17.5	1.45 ± 0.26	74.7
Control[e]	1.94 ± 0.41	100.0	1.94 ± 0.41	100.0

[a] Effector cells were stimulated with autologous, irradiated FSF expressing FIV capsid for 7 days. The supernatants collected from the stimulated cells on the seventh day were ultracentrifuged and added at a medium to supernatant ratio of 1:1 to FIV-PPR-infected target cells every 3 days.
[b] Effector cells were stimulated with autologous, irradiated FSF not expressing the FIV capsid for 7 days. The supernatants collected from the stimulated cells on the seventh day were ultracentrifuged and added at a medium to supernatant ratio of 1:1 to FIV-PPR-infected target cells every 3 days.
[c] FIV expression was determined by use of an FIV capsid antigen detection ELISA as mentioned in the Materials and methods.
[d] Relative to virus expression of untreated control cells.
[e] These control cells were infected with FIV-PPR and cultured in complete medium without effector cell supernatant.
* Statistically significant at a P value of <0.05.

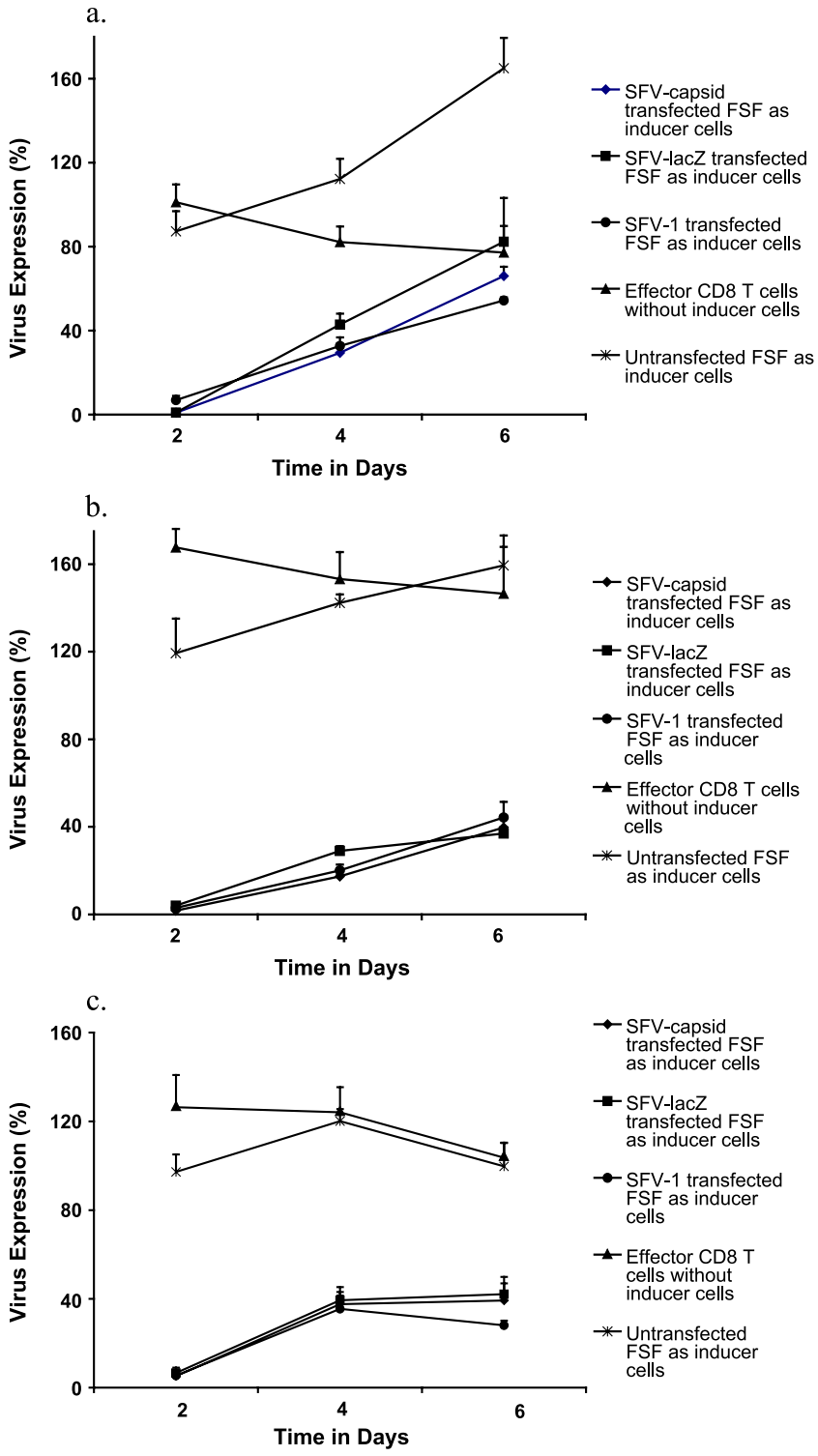

Fig. 1. Kinetics of the induction process using $CD8^+$ T lymphocytes as effector cells when FSF were used as inducer cells. FSF prepared from cat AZV2 were transfected with SFV vector expressing FIV capsid, an irrelevant antigen such as lacZ or SFV vector expressing only its polymerase, or left untransfected. $CD8^+$ T lymphocytes enriched from fresh PBMC from FIV-infected cats AZV2 (a), AUO2 (b) and FIV-uninfected cat AUS3 (c) were overlaid on FSF. Every 2 days, the supernatants were collected and stored at 4 °C and the non-adherent cells were overlaid on newly transfected FSF. As controls, supernatants were collected from untransfected FSF cultured without $CD8^+$ T lymphocytes, $CD8^+$ T lymphocytes cultured in the absence of FSF and $CD8^+$ T lymphocytes co-cultured with untransfected FSF. The supernatants were ultracentrifuged and added to FIV-PPR-infected target cells (cat OAE5 PBMC) every 3 days at a medium to supernatant ratio of 1:1. FIV production was determined by use of an FIV-capsid antigen detection ELISA. All the values have been normalized with respect to values obtained for untransfected FSF cultured without $CD8^+$ T lymphocytes.

within the PBMC contributed to the suppression of FIV replication in vitro. This population of $CD8^+$ T lymphocytes contained not more than 5% macrophages and not more than 10% $CD4^+$ T lymphocytes as determined by flow cytometry (data not shown). The kinetics of the soluble anti-FIV activity was determined using cat AZV2 FSF as inducer cells and enriched $CD8^+$ T lymphocytes from FIV-infected cats (AZV2 and AUO2) and an FIV-uninfected cat (AUS3) as effector cells. Supernatants were collected every 2 days from the co-culture of FSF transfected with SFV vector expressing FIV capsid, and autologous or heterologous $CD8^+$ T lymphocytes. In the presence of these FSF, supernatants collected on days 2 and 4 demonstrated the greatest suppression for the three cats examined. Similar results were obtained when fresh PBMC were used as effector cells (data not shown). However, in contrast to studies using PBMC as a source of effector cells, when $CD8^+$ T cells were used as effector cells, suppression of FIV replication was not only observed on day 6, but greater suppression was observed using supernatants collected on days 2 and 4 of induction (Figs. 1a–c). The presence of macrophages and/or $CD4^+$ T cells may either dilute the suppression or actively inhibit the suppressive activity of $CD8^+$ T effector cells.

Interestingly, following induction with FSF transfected with SFV vector expressing an irrelevant antigen such as lacZ or SFV vector by itself, supernatants from effector $CD8^+$ T cells of infected cats (AZV2 and AUO2) and an uninfected cat (AUS3) were also able to suppress FIV replication in vitro (Figs. 1a–c). This was also observed when PBMC were used as effector cells (data not shown). SFV vector without an insert expresses only the polymerase gene of the SFV during the course of transcription. It is possible that the polymerase or the viral RNA stimulates the effector cells, demonstrating that this kind of induction is not specific for FIV. Supernatants obtained from non-transfected FSF were not able to suppress FIV replication on any of the days examined. Supernatants from effector $CD8^+$ T cells cultured without FSF did not demonstrate any suppressing activity on any of the days examined (Figs. 1a–c). In addition, supernatants obtained from $CD8^+$ T cells co-cultured with non-transfected FSF did not show any suppressing activity (Figs. 1a–c). Thus, again non-transfected FSF cells could not induce $CD8^+$ T cells to produce anti-FIV activity.

Maximum suppression of FIV replication is dependent on inducer cell–effector cell contact

Transwell cell culture plates were used to examine whether cell contact-mediated stimulation was necessary for the production of the soluble anti-FIV activity. Suppression was consistently lower, although never eliminated, when a 0.45-μm membrane in transwell dishes separated the autologous irradiated inducer T cells and the effector cells. When supernatants collected on day 7 from effector cells in contact with the inducer cells were added to FIV-PPR-infected target cells, all supernatants with the exception of supernatant from cat E238 (at 2 years post-infection), demonstrated strong suppression of virus replication (Table 4). Presently, at 4 years post-infection, effector cells from cat E238 are able to suppress replication of FIV in vitro (unpublished data). When supernatants collected on day 7 from effector cells without direct contact with inducer cells were added to target cells, half suppressed virus replication (FIV-infected cats AUO3, AWF1 and AZV2); however, supernatants from the remaining FIV-infected cats E238, E284 and OLQ5 did not (Table 4). Overall, the amount of suppression of FIV replication observed with the supernatants collected when the inducer and effector cells were in contact with each other was reduced by approximately 50% when the inducer and effector cells were separated in a transwell plate. Therefore, inducer cell contact was required for optimal stimulation of effector cells for production of the soluble anti-FIV activity.

The kinetics of the induction process using transwell culture plates indicated that the supernatants obtained from the co-culture of the autologous irradiated inducer T cells and the effector cells in contact with each other were able

Table 4
Effect of cell contact between effector cells and inducer cells on induction of the soluble anti-FIV activity

Cats	Viral antigen expression in supernatants from target cells that received supernatants from effector cells which were			
	In contact with inducer T cells[a]		Not in contact with inducer T cells[b]	
	Capsid OD[c]	% Virus expression[d]	Capsid OD	% Virus expression
FIV (+)				
AUO3	0.13 ± 0.01*	4.3	0.28 ± 0.02*	9.2
AWF1	0.51 ± 0.03*	16.8	0.98 ± 0.14*	32.2
AZV2	0.77 ± 0.02*	25.3	1.61 ± 0.31*	53.0
E238	3.90 ± 0.07	101.6	3.78 ± 0.21	124.3
E284	1.91 ± 0.55*	62.8	3.88 ± 0.13	127.6
OLQ5	0.41 ± 0.13*	13.5	3.75 ± 0.12	123.4
Control[e]	3.04 ± 0.61	100.0	3.04 ± 0.61	100.0

[a] Effector cells were co-cultured with FIV-infected irradiated autologous inducer T cells such that they were in contact with each other. Cell culture supernatants collected after 7 days were ultracentrifuged and added at a medium to supernatant ratio of 1:1 to FIV-PPR-infected target cells every 3 days.
[b] Effector cells were co cultured with FIV-infected irradiated autologous inducer T cells such that they were separated by a 0.45-μm membrane in a transwell plate. Cell culture supernatants collected after 7 days were ultracentrifuged and added at a medium to supernatant ratio of 1:1 to FIV-PPR-infected target cells every 3 days.
[c] FIV expression was determined by an FIV capsid antigen detection ELISA as mentioned in the Materials and methods.
[d] Relative to virus expression of untreated control cells.
[e] These control cells were infected with FIV-PPR and cultured without effector cell supernatant.
* Statistically significant at a P value of <0.05.

to suppress FIV replication on the third day (Fig. 2a) but this suppression was lost by the sixth day (Fig. 2b) for FIV-infected cats AZV2, AUO2 and FIV-uninfected cat AUS3. However, when the inducer cells and the effector cells from FIV-infected cats AZV2 and AUO2 were separated by a 0.45-μm membrane, the amount of suppression of virus replication seen on the third day was again reduced by nearly half of that seen when the inducer and the effector cells were in contact with each other. Effector cell supernatant from FIV-uninfected cat AUS3 was able to suppress FIV replication on either day 3 or day 6, but only marginally as compared with the infected cats. The supernatants from the PBMC cultured in the absence of inducer cells did not suppress FIV replication either on day 3 or day 6 for any of the cats examined (Figs. 2a and 2b). Thus, under the conditions where FSF were used as inducer cells or when FIV-infected inducer T cells were separated from the effector cells, the optimal days for the induction of the soluble anti-FIV activity continued until the fourth day of induction, after which a decrease in the anti-FIV activity was consistently seen.

Discussion

These studies suggest that more than one pattern of induction stimulates antiviral activity in our chronically FIV-infected and -uninfected control cats. Studies performed in our laboratory consistently indicate that it is the $CD8^+$ T cells within the population of PBMC that are responsible for the soluble anti-FIV activity (Choi et al., 2000; Figs. 1a–1c). The various patterns of induction may stimulate several molecules that mediate $CD8^+$ T-cell suppressive activity against FIV. It is likely that the mechanism of induction differs quantitatively and maybe even to a greater extent, qualitatively, leading to the induction of distinct antiviral factors. Moriuchi et al. (1996) have demonstrated that the HIV suppressor activity of $CD8^+$ T-cell supernatants is multifactorial and that various factors within these supernatants including, but not limited to, the β-chemokines, may affect HIV replication at various stages of the life cycle of the virus. Due to the distinct $CD8^+$ T-cell suppressor activities that have been reported from various laboratories, Copeland (2002) has also suggested that the CAF activity in humans could

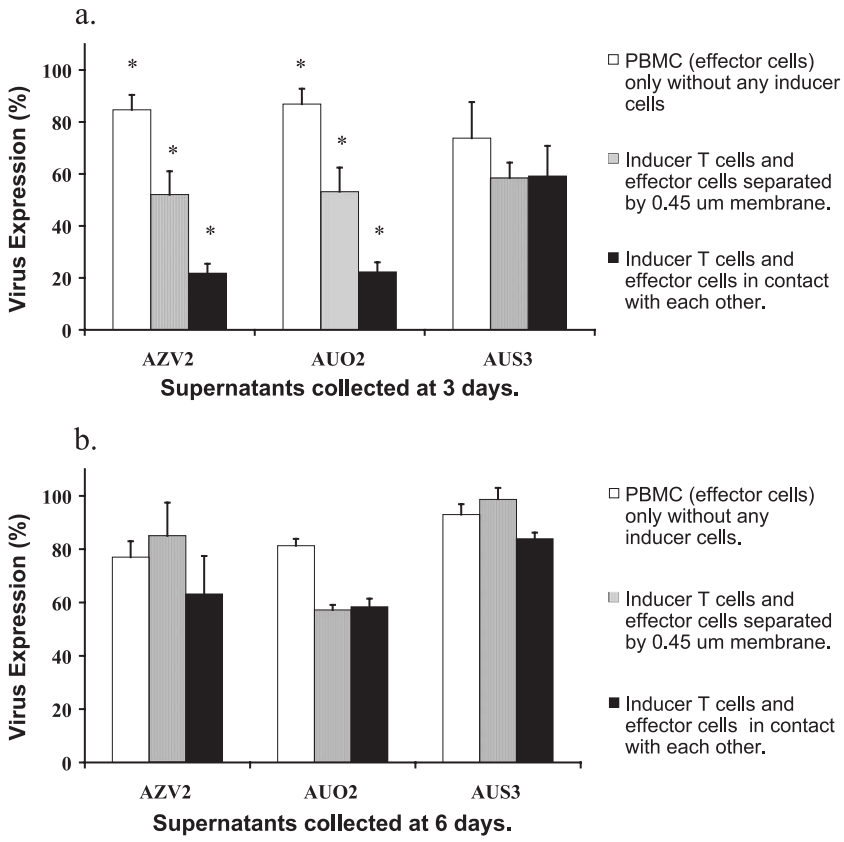

Fig. 2. Kinetics of inducer cell–effector cell contact for the induction process. Fresh PBMC (effector cells) from FIV-infected cats AZV2, AUO2 and uninfected cat AUS3 were co-cultured in contact with autologous irradiated FIV-infected inducer T cells or separated from the inducer T cells by a 0.45-μm membrane. Cell culture supernatants collected on days 3 (a) and 6 (b) were ultracentrifuged and added to FIV-PPR-infected target cells (cat OAE5 PBMC) every 3 days at a medium to supernatant ratio of 1:1. FIV production was determined by use of an FIV capsid antigen detection ELISA. Values indicated by asterisks are significant (as calculated by the *t* test) among the three parameters examined, at 3 days for FIV-infected cats AZV2 and AUO2.

represent more than one factor. Thus, it is possible that the various modes of induction lead to the secretion of several molecules mediating the $CD8^+$ T-cell suppressive activity against FIV, and by inference, HIV. Suppression of FIV replication in our cats likely depends on the distinct patterns of induction resulting from the differences in the innate immune system of the cats, the course of infection and illness, and the differences in the genetics of the cats. The levels of suppressing activity generated have remained highly reproducible for each animal over several years, although variable from cat to cat. Interestingly, at 2 years post-infection, lymphocytes from cat E238, infected with the highest dose of FIV-PPR (1250 $TCID_{50}$), did not produce detectable soluble anti-FIV activity (Table 3), but had the highest levels of CTL activity (Choi et al., 2000). Presently, E238, after more than 4 years of infection, is able to suppress replication of FIV (unpublished data). All the FIV-infected cats in our colony have been infected between 3 and 5 years and are in the chronic asymptomatic stage of infection. The proviral load of these cats has been found to inversely correlate with their ability to suppress FIV replication in vitro (manuscript under preparation). It is possible that the suppressing activity of the $CD8^+$ T cells actually helps to maintain the asymptomatic nature of the cats, because as yet, we have been unable to demonstrate anti-FIV $CD8^+$ T-cell-suppressing activity in acutely infected cats. The various types of cells that function in the induction of the effector cells and the communication required for the induction of the antiviral activity against FIV or HIV have not yet been defined. The focus of this study was to determine the requirements for induction of suppression of FIV replication, specifically whether T cells were necessary for induction of the feline $CD8^+$ T-cell-suppressing activity and whether FIV infection was a criterion for cells to function as inducer cells. Inducer cells used in our initial studies were irradiated FIV-infected T lymphocytes (Choi et al., 2000). But immortalized FSF, transfected with SFV vector expressing either the FIV capsid or an irrelevant antigen, such as lacZ or FSF transfected with SFV vector expressing only its polymerase, could also mediate the induction. Either irradiated or non-irradiated FSF could serve as inducer cells. The use of FSF transfected with SFV vector expressing the capsid protein of the FIV-PPR strain might serve as a convenient alternative to the use of infected T cells as inducer cells in our system. This is the first published report of FSF, transfected with SFV-capsid, SFV-lacZ or SFV only, serving as inducer cells to stimulate the soluble anti-FIV activity. Thus, it appears that functional inducer cells do not need to be professional antigen presenting cells (APC) and these cells do not have to express the viable and complete virus.

The effector cells produced the soluble anti-FIV activity in the absence of MHC-matched inducer cells when either FIV-infected T cells or transfected FSF were used. This has been shown in our studies using autologous or heterologous inducer cells to stimulate the effector cells. This is in contrast to the MHC class I-restricted, FIV-specific cytotoxic responses described for $CD8^+$ T cells from our FIV-infected cats, where autologous FIV-infected T cells were used as APC (Song et al., 1992). The fact that supernatants from the co-culture of effector cells (from FIV-uninfected cat AUS3 and FIV-infected cat AUO3) and mismatched inducer T cells were able to suppress FIV to a greater extent is in accordance with our studies using FSF as inducer cells. In the studies with FSF, we have consistently shown that supernatants from effector cells (either PBMC—data not shown or $CD8^+$ T cells—Figs. 1a–1c) when co-cultured with mismatched FSF transfected with SFV vector expressing either the FIV capsid or an irrelevant antigen, such as lacZ or FSF transfected with SFV vector expressing only its polymerase, suppress FIV replication. In our studies, non-transfected FSF alone do not induce effector cells and although MHC restriction is not required for the soluble anti-FIV response, it is not yet clear as to whether it might improve or otherwise contribute to the activation of the effector cells.

Optimal induction of the suppressing activity from the effector cells depended on cell–cell contact between inducer and effector cells, although approximately 50% of the suppressing activity could be demonstrated when the inducer and the effector cells were not in contact with each other. Therefore, membrane antigen interactions may be critical in the communication, while cytokines may play an accessory role in the induction process.

The kinetics of the induction process demonstrated that antiviral activity against FIV was maximally expressed until day 4 after induction, but it was reduced appreciably or was absent by the sixth day. However, suppression by cell culture supernatants collected on the seventh day indicates that accumulation of antiviral factors from day 1 to day 7 contributed to suppression of FIV replication when accumulative supernatants were collected on day 7. This demonstrates that the antiviral activity is stable over the 7-day period. Thus, understanding the mechanisms that activate and control suppression of FIV may lead to our understanding of the natural process for controlling chronic lentiviral infection.

FIV-infected T cells and FSF transfected with SFV vector expressing the FIV capsid, an irrelevant antigen such as lacZ or FSF transfected with SFV vector only, could be used to induce the effector cells to suppress FIV replication in vitro. Both FSF and FIV-infected T cells would seem to require communication of some kind of crisis to induce the effector cells. Identifying alterations occurring with respect to membrane antigen expression within these two types of inducer cells will be valuable in understanding conditions needed for induction of effector cell synthesis of antiviral activity. Characterization and identification of the stimuli and mechanisms involved in the induction of the antiviral activity against FIV, not addressed in other studies, will

provide information for strategies of practical exploitation of this innate T-cell immunity and may contribute to the design of novel, safe and complementary anti-FIV therapeutic strategies.

Materials and methods

Experimental animals

Specific, pathogen-free cats purchased from Harlan Sprague–Dawley, Madison, WI, or Liberty Laboratories, Liberty Corner, NJ, were serologically negative for feline leukemia virus. Cats were housed in a specific, pathogen-free environment at the Laboratory Animal Research and Resources Facility, Texas A&M University, College Station, TX. Cats AUO2, AUO3, AWF1, AZV2, OLQ5, OLM6, E238 and E284 were chronically infected with FIV-PPR strain. From 3 to 5 years previously, 50–1250 $TCID_{50}$ of virus had been administered i.v. Cats AUS3, OAE5 and OLQ4, sham inoculated with saline solution, were used as negative control cats.

Virus

FIV-PPR was propagated in feline PBMC. After 7–10 days of infection, virus replication was evaluated with an FIV capsid antigen detection ELISA (Choi et al., 2000). Supernatants with an OD of more than 2 were collected and these stocks were stored at −80 °C. The FIV-PPR virus stock had a titer of $0.5 \times 10^{5.1}$ in these experiments, determined as described by Hokanson et al. (2000).

Cell culture

Feline PBMC were isolated from EDTA (K_3)-treated whole blood by Histopaque-1077 (Sigma, St. Louis, MO) density gradient centrifugation (Choi et al., 2000). PBMC were cultured as described previously with RPMI 1640 (Gibco BRL, Grand Island, NY) supplemented with 10% heat-inactivated fetal bovine serum (FBS) (Atlas Biologicals, Fort Collins, CO), 50 μg/ml of gentamicin (Gibco BRL), 5×10^{-5} M 2-mercaptoethanol (Gibco BRL), 2 mM L-glutamine (Gibco BRL), and 100 units of human recombinant IL-2 (hr Il-2) (Gibco BRL) per milliliter (Choi et al., 2000). Cells were grown at 37 °C in a humidified atmosphere of 5% CO_2.

Continuous culture of FSF

To develop FSF cell lines, tissues were prepared from skin plug biopsies of infected cats AUO2, AUO3, AWF1, AZV2, OLQ5 and uninfected cat OAE5. After five to six passages in culture, the cells were transformed by transfection of SV40 T antigen-expressing plasmid DNA (Gift from David Busbee, Department of Veterinary Anatomy, Texas A&M University). The transformed FSF were selected by adding geneticin (0.5 mg/ml of the medium) (Gibco BRL). The FSF were cultured in MEM-alpha supplemented by 10% heat-inactivated FBS (Atlas Biologicals), 2 mM L-glutamine (Gibco BRL), 20 units/ml of epidermal growth factor (Gibco BRL) and 50 μg/ml of gentamicin (Gibco BRL).

Preparation of FIV-PPR-infected inducer T cells

Feline T cells were infected with FIV-PPR strain and irradiated (11,000 rads from a ^{60}Co source provided by the Nuclear Science Center, Texas Engineering Experiment Station, College Station, TX) before using as inducer cells for the stimulation of effector cells, as previously described (Choi et al., 2000; Song et al., 1992). Briefly, freshly prepared PBMC were stimulated for 3 days with 5 μg/ml of Con A (Sigma). The stimulated cells were infected with FIV-PPR for 1 h at 37 °C. The cells were washed and cultured in complete RPMI containing 100 units hr Il-2 per milliliter. After 6 days of incubation, virus replication was monitored using the FIV capsid antigen ELISA. These FIV-infected cells were cryo-preserved to be used later as inducer cells.

Preparation of FSF as inducer cells

The gene encoding FIV capsid antigen was amplified by PCR from FIV-PPR-infected T cells using the following primers synthesized with *Bam*HI sites at the 5′ends; forward primer: 5′CGG GAT CCA CAA ACA GTA AAT GGA GC-3′ and backward primer: 5′-CGG GAT CCT ATC TCT TGA CAA GC-3′. The PCR conditions used were 30 s at 94 °C, 30 s at 55 °C and 45 s at 72 °C for 30 cycles. The PCR product was directly cloned into the TOPO TA cloning vector (Invitrogen, Carlsbad, CA). The cloned DNA with the correct orientation for capsid expression was digested by the *Bam*HI restriction enzyme. The digested DNA was cleaned by gel extraction kit (Qiagen, Valencia, CA) and ligated into *Bam*HI-digested SFV vector (Gibco BRL). SFV-capsid recombinant virus particles were made by cotransfection of SFV-capsid recombinant mRNA and SFV-helper-2 mRNA into BHK-21 cells according to the SFV gene expression system instruction manual (Gibco BRL). FSF were infected with SFV-capsid recombinant particles or transfected with the SFV-capsid, SFV-lacZ and SFV RNA. The SFV vector RNA was prepared by transcribing in vitro from *Spe*I-linearized recombinant vectors and transfecting into FSF using DMRIE-C reagent (Gibco BRL). After 18 h in complete RPMI, the SFV capsid-expressing FSF were used as inducer cells.

Phenotype enrichment

Negative selection for enrichment of $CD8^+$ T from freshly isolated PBMC of FIV-PPR-infected cats was performed using a combination of panning and magnetic beads.

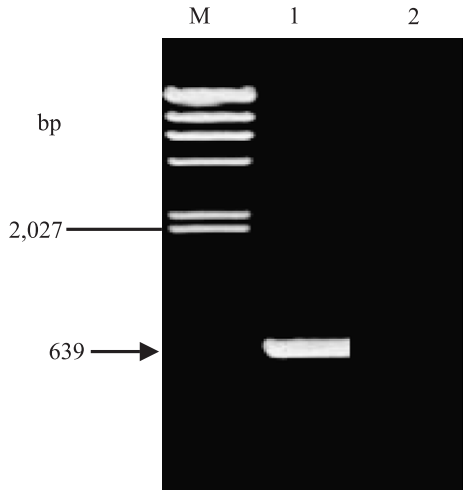

Fig. 3. Detection of the FIV capsid sequence in infected FSF by RT-PCR analysis. FSF were infected with SFV capsid recombinant particles or left uninfected. cDNA was made from the RNA from the infected and the uninfected cells and amplified by PCR. PCR analysis was performed using primers specific for FIV capsid (forward primer 5′-CGGGATCCACAAACAGTAAATGGAGC-3′ and backward primer 5′-CGGGATCCTATCTCTTGACAAGC-3′) using cDNA from infected cells (lane 1) or uninfected cells (lane 2). The arrow indicates the capsid-specific 639-bp PCR product in lane 1. DNA marker (Lambda DNA/*Hin*dIII, Promega, Madison, WI) is indicated in lane M.

Briefly, plastic adherent cells were first removed from fresh PBMC by incubating the PBMC in complete RPMI at 37°C in a humidified atmosphere of 5% CO_2 for 4 h. The non-adherent cells were washed once in phosphate-buffered saline (PBS). The washed cells were then incubated in 1 ml of PBS with 3% FBS containing 500 μl of anti-feline CD4 monoclonal antibody (CAT30A, gift from Dr. Wayne Tompkins, North Carolina State University) and 30 μl of mouse anti-human CD14 monocyte antibody (Clone TUK4, Dako, Carpinteria, CA) for 30 min at 4°C on a mixer. The cells were washed once in PBS, resuspended in 1 ml of PBS with 3% FBS containing M450 dynabeads that had been coated with goat anti-mouse IgG (Dynal Biotech, Great Neck, NY) and incubated for 30 min at 4°C on a mixer. After the bead-bound cells ($CD4^+$ T cells and $CD14^+$ cells) were magnetically removed, the remaining cells, mostly $CD8^+$ T cells, were resuspended in 1 ml of PBS with 3% FBS, gently agitated to release any trapped nonbound cells, and then magnetically reisolated. The purity of the $CD8^+$ T cells was confirmed by flow cytometry (data not shown).

Stimulation of effector cells by SFV capsid-expressing inducer cells

At an effector to inducer cell ratio of 20:1, autologous irradiated FSF infected with SFV-capsid recombinant virus particles were added to effector cells from FIV-infected cats AUO2, AUO3, AWF1, AZV2 and OLQ5 and FIV-uninfected cat OAE5 and medium was changed every 3 or 4 days. Expression of capsid antigen on FSF was detected by RT-PCR that amplified and detected the FIV capsid gene (Fig. 3). After 7 days of stimulation, cell culture supernatants were collected, stored at 4°C and the FIV-suppressive activity of the supernatants was tested as described below.

Alternatively, FSF prepared from cat AZV2 were plated in six-well plates (3×10^5 cells/well) and were transfected with SFV-capsid RNA, SFV lacZ RNA or SFV-1 RNA (vector only without any insert) using DMRIE-C (Gibco BRL). Five micrograms of RNA was used for each transfection reaction. Expression of SFV-capsid on the FSF was confirmed by an immunofluorescence assay (IFA) as described elsewhere (Brown et al., 1991) (Fig. 4). Expression of lacZ was confirmed by adding X-galactosidase (Promega). These inducer cells transfected with SFV-capsid RNA, SFV-lacZ RNA or SFV RNA were overlaid 18 h after transfection with autologous or heterologous effector cells (either fresh PBMC—data not shown or $CD8^+$ T cells—Figs. 1a–1c) from FIV-infected and -uninfected cats. Every 2 days for 6 days, the non-adherent cells were harvested and centrifuged at 1200 rpm for 10 min (Jouan, Winchester, VA), and the supernatants collected and stored at 4°C before overlaying the cells onto freshly prepared inducer cells. Controls were non-transfected FSF cultured in the absence of effector cells, effector cells cultured without FSF and

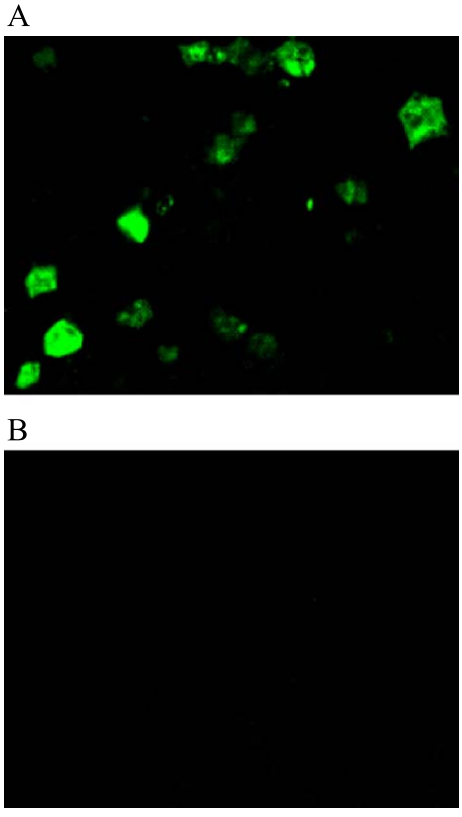

Fig. 4. Immunofluorescent staining of the FIV capsid protein expressed on FSF. FIV capsid protein expressed on FSF after transfection of FSF with SFV vector expressing FIV capsid RNA (A). Untransfected FSF cells are shown in B. Magnification, ×200.

non-transfected FSF co-cultured with effector cells. The supernatants collected from these cells on days 2, 4 and 6 were stored at 4°C.

Transwell separation of cells

To assess the role of cell–cell contact in the induction of the effector cells, the irradiated inducer T cells were co-cultured with autologous effector PBMC in six-well tissue culture plates in which the two cell populations were separated by a 0.45-μm pore size polycarbonate membrane (Corning Incorporated, Acton, MA) at a ratio of 1:5 inducer to effector cells in a total volume of 3 ml of complete RPMI. Inducer cells were also co-cultured with the effector PBMC, such that the inducer cells and the effector cells were in contact with each other. PBMC cultured without any inducer cells were used as controls. Newly irradiated inducer cells were added to the effector cells on the third day. The supernatants were collected on days 3, 6 or 7 and stored at 4°C.

FIV suppression assay

Cell-free supernatants stored at 4°C were ultracentrifuged at 35,000 rpm for 2 h (Beckman L7-55 Ultracentrifuge, Palo Alto, CA) to remove the residual viral particles. The supernatants were passed through 0.22-μm-pore size filters (Pall Corporation, Ann Arbor, MI) and stored at 4°C. Target cells were prepared from PBMC of an FIV-uninfected cat after culturing in vitro for 6 days with Con A and IL-2 (Choi et al., 2000). They were then infected with the FIV-PPR strain and cultured with supernatants in a 96-well plate for 10 days at a medium to supernatant ratio of 1:1. Supernatant and fresh complete RPMI + hr Il-2 100 units/ml was added to the cells every 3 days. The amount of FIV in the supernatants was determined by an FIV capsid antigen detection ELISA. Suppression was considered to be positive when virus expression was ≤65% of virus expression in the untreated controls. Positive controls were FIV-infected PBMC cultured in complete RPMI in the absence of effector cell supernatants.

Statistics

The differences in FIV replication between infected control cells and cells in which suppression of FIV was seen were analyzed with a two-tailed Student's *t* test (Ott, 1993). Statistically significant differences were set at a *P* value of <0.05.

Acknowledgments

We thank Anna Mata, Lynda Case, Angel Tapia, Carrie Giordano and Kelly Frame for collection of feline blood samples. We also thank Dr. Mary Carpino for her help with collection of the skin plug biopsies from the cats and Dr. Roger Smith for his assistance with flow cytometry. In addition, we acknowledge the Nuclear Science Center at Texas A&M University for irradiation of inducer cells. This work was funded by National Institute of Allergy and Infectious Diseases grant AI 32360-01, by Center for AIDS Research grant 502342, by Morris Animal Foundation grant 96FE-09 and by Dr. Ann Kier, department head of the Department of Veterinary Pathobiology, Texas A&M University.

References

Appay, V., Nixon, D.F., Donahoe, S.M., Gillespie, G.M., Dong, T., King, A., Ogg, G.S., Spiegel, H.M., Conlon, C., Spina, C.A., Havlir, D.V., Richman, D.D., Waters, A., Easterbrook, P., McMichael, A.J., Rowland-Jones, S.L., 2000. HIV-specific CD8 (+) T cells produce antiviral cytokines but are impaired in cytolytic function. J. Exp. Med. 192 (1), 63–75.

Barker, E., 1999. CD8+ cell-derived anti-human immunodeficiency virus inhibitory factor. J. Infect. Dis. 179 (Suppl. 3), S485–S488.

Barouch, D.H., Letvin, N.L., 2001. CD8+ cytotoxic T lymphocyte responses to lentiviruses and herpesviruses. Curr. Opin. Immunol. 13 (4), 479–482.

Blackbourn, D.J., Chuang, L.F., Killam Jr., K.F., Chuang, R.Y., 1994. Inhibition of simian immunodeficiency virus (SIV) replication by CD8+ cells of SIV-infected rhesus macaques: implications for immunopathogenesis. J. Med. Primatol. 23 (6), 343–354.

Blackbourn, D.J., Mackewicz, C.E., Barker, E., Hunt, T.K., Herndier, B., Haase, A.T., Levy, J.A., 1996. Suppression of HIV replication by lymphoid tissue CD8+ cells correlates with the clinical state of HIV-infected individuals. Proc. Natl. Acad. Sci. U.S.A. 93 (23), 13125–13130.

Brinchmann, J.E., Gaudernack, G., Vartdal, F., 1990. CD8+ T cells inhibit HIV replication in naturally infected CD4+ T cells. Evidence for a soluble inhibitor. J. Immunol. 144 (8), 2961–2966.

Brown, W.C., Bissey, L., Logan, K.S., Pedersen, N.C., Elder, J.H., Collisson, E.W., 1991. Feline immunodeficiency virus infects both $CD4^+$ and $CD8^+$ T lymphocytes. J. Virol. 65 (6), 3359–3364.

Bucci, J.G., English, R.V., Jordan, H.L., Childers, T.A., Tompkins, M.B., Tompkins, W.A., 1998a. Mucosally transmitted feline immunodeficiency virus induces a CD8+ antiviral response that correlates with reduction of cell-associated virus. J. Infect. Dis. 177 (1), 18–25.

Bucci, J.G., Gebhard, D.H., Childers, T.A., English, R.V., Tompkins, M.B., Tompkins, W.A., 1998b. The CD8+ cell phenotype mediating antiviral activity in feline immunodeficiency virus-infected cats is characterized by reduced surface expression of the CD8 beta chain. J. Infect. Dis. 178 (4), 968–977.

Charaf, B., Sanhadji, K., Sekkat, S., Farouqui, B., Touraine, J.L., Benslimane, A., 1993. MHC-I non-restricted cytotoxic activity in *Macaca sylvana* experimentally inoculated with HIV2 and SIV/mac. Thymus 22 (1), 1–12.

Choi, I.S., Hokanson, R., Collisson, E.W., 2000. Anti-feline immunodeficiency virus (FIV) soluble factor(s) produced from antigen-stimulated feline CD8(+) T lymphocytes suppresses FIV replication. J. Virol. 74 (2), 676–683.

Clerici, M., Balotta, C., Galli, M., 1996. Soluble HIV suppressive factors: more than one Holy Grail? Immunol. Today 17 (7), 297–298.

Copeland, K.F., 2002. The role of CD8+ T cell soluble factors in human immunodeficiency virus infection. Curr. Med. Chem. 9 (20), 1781–1790.

Copeland, K.F., Leith, J.G., McKay, P.J., Kelleher, L., Smaill, F.M., Rosenthal, K.L., 1997. CD8+ T cell-mediated suppression of HIV long ter-

minal repeat-driven gene expression is not associated with improved clinical status. AIDS 11 (5), 581–586.
Crawford, P.C., Papadi, G.P., Levy, J.K., Benson, N.A., Mergia, A., Johnson, C.M., 2001. Tissue dynamics of CD8 lymphocytes that suppress viral replication in cats infected neonatally with feline immunodeficiency virus. J. Infect. Dis. 184 (6), 671–681.
Flynn, J.N., Cannon, C.A., Sloan, D., Neil, J.C., Jarrett, O., 1999. Suppression of feline immunodeficiency virus replication in vitro by a soluble factor secreted by CD8+ T lymphocytes. Immunology 96 (2), 220–229.
Flynn, J.N., Dunham, S., Mueller, A., Cannon, C., Jarrett, O., 2002. Involvement of cytolytic and non-cytolytic T cells in the control of feline immunodeficiency virus infection. Vet. Immunol. Immunopathol. 85 (3–4), 159–170.
Furci, L., Lopalco, L., Loverro, P., Sinnone, M., Tambussi, G., Lazzarin, A., Lusso, P., 2002. Non-cytotoxic inhibition of HIV-1 infection by unstimulated CD8+ T lymphocytes from HIV-exposed-uninfected individuals. AIDS 16 (7), 1003–1008.
Gebhard, D.H., Dow, J.L., Childers, T.A., Alvelo, J.I., Tompkins, M.B., Tompkins, W.A., 1999. Progressive expansion of an L-selectin-negative CD8 cell with anti-feline immunodeficiency virus (FIV) suppressor function in the circulation of FIV-infected cats. J. Infect. Dis. 180 (5), 1503–1513.
Geiben-Lynn, R., Kursar, M., Brown, N.V., Kerr, E.L., Luster, A.D., Walker, B.D., 2001. Noncytolytic inhibition of X4 virus by bulk CD8(+) cells from human immunodeficiency virus type 1 (HIV-1)-infected persons and HIV-1-specific cytotoxic T lymphocytes is not mediated by beta-chemokines. J. Virol. 75 (17), 8306–8316.
Gomez, A.M., Smaill, F.M., Rosenthal, K.L., 1994. Inhibition of HIV replication by CD8+ T cells correlates with CD4 counts and clinical stage of disease. Clin. Exp. Immunol. 97, 68–75.
Hohdatsu, T., Okubo, M., Koyama, H., 1998. Feline CD8+ T cell noncytolytic anti-feline immunodeficiency virus activity mediated by a soluble factor(s). J. Gen. Virol. 79 (Pt 11), 2729–2735.
Hohdatsu, T., Miyagawa, N., Ohkubo, M., Kida, K., Koyama, H., 2000. Studies on feline CD8+ T cell non-cytolytic anti-feline immunodeficiency virus (FIV) activity. Arch. Virol. 145 (12), 2525–2538.
Hohdatsu, T., Sasagawa, T., Yamazaki, A., Motokawa, K., Kusuhara, H., Kaneshima, T., Koyama, H., 2002. CD8+ T cells from feline immunodeficiency virus (FIV) infected cats suppress exogenous FIV replication of their peripheral blood mononuclear cells in vitro. Arch. Virol. 147 (8), 1517–1529.
Hokanson, R.M., TerWee, J., Choi, I.S., Coates, J., Dean, H., Reddy, D.N., Wolf, A.M., Collisson, E.W., 2000. Dose response studies of acute feline immunodeficiency virus PPR strain infection in cats. Vet. Microbiol. 76 (4), 311–327.
Hsueh, F.W., Walker, C.M., Blackbourn, D.J., Levy, J.A., 1994. Suppression of HIV replication by CD8+ cell clones derived from HIV-infected and uninfected individuals. Cell. Immunol. 159 (2), 271–279.
Jeng, C.R., English, R.V., Childers, T., Tompkins, M.B., Tompkins, W.A., 1996. Evidence for CD8+ antiviral activity in cats infected with feline immunodeficiency virus. J. Virol. 70 (4), 2474–2480.
Kannagi, M., Chalifoux, L.V., Lord, C.I., Letvin, N.L., 1988. Suppression of simian immunodeficiency virus replication in vitro by CD8+ lymphocytes. J. Immunol. 140 (7), 2237–2242.
Kootstra, N.A., Miedema, F., Schuitemaker, H., 1997. Analysis of CD8+ T lymphocyte-mediated nonlytic suppression of autologous and heterologous primary human immunodeficiency virus type 1 isolates. AIDS Res. Hum. Retrovir. 13 (8), 685–693.
Landay, A.L., Mackewicz, C.E., Levy, J.A., 1993. An activated CD8+ T cell phenotype correlates with anti-HIV activity and asymptomatic clinical status. Clin. Immunol. Immunopathol. 69 (1), 106–116.
Le Borgne, S., Fevrier, M., Callebaut, C., Lee, S.P., Riviere, Y., 2000. CD8(+)-cell antiviral factor activity is not restricted to human immunodeficiency virus (HIV)-specific T cells and can block HIV replication after initiation of reverse transcription. J. Virol. 74 (10), 4456–4464.
Levy, J.A., 2001. The importance of the innate immune system in controlling HIV infection and disease. Trends Immunol. 22 (6), 312–316.
Levy, J.A., Mackewicz, C.E., Barker, E., 1996. Controlling HIV pathogenesis: the role of the noncytotoxic anti-HIV response of CD8+ T cells. Immunol. Today 17 (5), 217–224.
Levy, J.A., Hsueh, F., Blackbourn, D.J., Wara, D., Weintrub, P.S., 1998. CD8 cell noncytotoxic antiviral activity in human immunodeficiency virus-infected and -uninfected children. J. Infect. Dis. 177 (2), 470–472.
Mackewicz, C.E., Ortega, H.W., Levy, J.A., 1991. CD8+ cell anti-HIV activity correlates with the clinical state of the infected individual. J. Clin. Invest. 87 (4), 1462–1466.
Mackewicz, C.E., Yang, L.C., Lifson, J.D., Levy, J.A., 1994. Non-cytolytic CD8 T-cell anti-HIV responses in primary HIV-1 infection. Lancet 344 (8938), 1671–1673.
Mackewicz, C.E., Garovoy, M.R., Levy, J.A., 1998. HLA compatibility requirements for CD8(+)-T-cell-mediated suppression of human immunodeficiency virus replication. J. Virol. 72 (12), 10165–10170.
Moriuchi, H., Moriuchi, M., Combadiere, C., Murphy, P.M., Fauci, A.S., 1996. CD8+ T-cell-derived soluble factor(s), but not beta-chemokines RANTES, MIP-1 alpha, and MIP-1 beta, suppress HIV-1 replication in monocyte/macrophages. Proc. Natl. Acad. Sci. U.S.A. 93 (26), 15341–15345.
Mosoian, A., Teixeira, A., Caron, E., Piwoz, J., Klotman, M.E., 2000. $CD8^+$ cell lines isolated from HIV-1-infected children have potent soluble HIV-1 inhibitory activities that differs from beta-chemokines. Viral Immunol. 13 (4), 481–495.
Ott, R.L., 1993. An Introduction to Statistical Methods and Data Analysis. Duxbury Press, Belmont, CA, pp. 197–258.
Pedersen, N.C., Ho, E.W., Brown, M.L., Yamamoto, J.K., 1987. Isolation of a T-lymphotropic virus from domestic cats with an immunodeficiency-like syndrome. Science 235 (4790), 790–793.
Pedersen, N.C., Yamamoto, J.K., Ishida, T., Hansen, H., 1989. Feline immunodeficiency virus infection. Vet. Immunol. Immunopathol. 21 (1), 111–129.
Pollack, H., Zhan, M.X., Safrit, J.T., Chen, S.H., Rochford, G., Tao, P.Z., Koup, R., Krasinski, K., Borkowsky, W., 1997. CD8+ T-cell-mediated suppression of HIV replication in the first year of life: association with lower viral load and favorable early survival. AIDS 11 (1), F9–F13.
Powell, J.D., Yehuda-Cohen, T., Villinger, F., McClure, H.M., Sell, K.W., Ahmed-Ansari, A., 1990. Inhibition of SIV/SMM replication in vitro by CD8+ cells from SIV/SMM infected seropositive clinically asymptomatic sooty mangabeys. J. Med. Primatol. 19 (3–4), 239–249.
Rosok, B., Voltersvik, P., Larsson, B.M., Albert, J., Brinchmann, J.E., Asjo, B., 1997. CD8+ T cells from HIV type 1-seronegative individuals suppress virus replication in acutely infected cells. AIDS Res. Hum. Retrovir. 13 (1), 79–85.
Song, W., Collisson, E.W., Billingsley, P.M., Brown, W.C., 1992. Induction of feline immunodeficiency virus-specific cytolytic T-cell responses from experimentally infected cats. J. Virol. 66 (9), 5409–5417.
Stranford, S.A., Skurnick, J., Louria, D., Osmond, D., Chang, S.Y., Sninsky, J., Ferrari, G., Weinhold, K., Lindquist, C., Levy, J.A., 1999. Lack of infection in HIV-exposed individuals is associated with a strong CD8(+) cell noncytotoxic anti-HIV response. Proc. Natl. Acad. Sci. U.S.A. 96 (3), 1030–1035.
Toso, J.F., Chen, C.H., Mohr, J.R., Piglia, L., Oei, C., Ferrari, G., Greenberg, M.L., Weinhold, K.J., 1995. Oligoclonal CD8 lymphocytes from persons with asymptomatic human immunodeficiency virus (HIV) type 1 infection inhibit HIV-1 replication. J. Infect. Dis. 172 (4), 964–973.
Walker, C.M., 1993. Non-cytolytic control of HIV replication by CD8+ T cells. Semin. Immunol. 5 (3), 195–201.
Walker, C.M., Moody, D.J., Stites, D.P., Levy, J.A., 1986. CD8+ lymphocytes can control HIV infection in vitro by suppressing virus replication. Science 234 (4783), 1563–1566.
Walker, C.M., Erickson, A.L., Hsueh, F.C., Levy, J.A., 1991a. Inhibition of human immunodeficiency virus replication in acutely infected CD4+ cells by CD8+ cells involves a noncytotoxic mechanism. J. Virol. 65 (11), 5921–5927.
Walker, C.M., Thomson-Honnebier, G.A., Hsueh, F.C., Erickson, A.L.,

Pan, L.Z., Levy, J.A., 1991b. CD8+ T cells from HIV-1-infected individuals inhibit acute infection by human and primate immunodeficiency viruses. Cell. Immunol. 137 (2), 420–428.

Yamamoto, J.K., Sparger, E., Ho, E.W., Andersen, P.R., O'Connor, T.P., Mandell, C.P., Lowenstine, L., Munn, R., Pedersen, N.C., 1988. Pathogenesis of experimentally induced feline immunodeficiency virus infection in cats. Am. J. Vet. Res. 49 (8), 1246–1258.

Yang, O.O., Kalams, S.A., Trocha, A., Cao, H., Luster, A., Johnson, R.P., Walker, B.D., 1997. Suppression of human immunodeficiency virus type 1 replication by CD8+ cells: evidence for HLA class I-restricted triggering of cytolytic and noncytolytic mechanisms. J. Virol. 71 (4), 3120–3128.

Zanussi, S., Simonelli, C., D'Andrea, M., Caffau, C., Clerici, M., Tirelli, U., DePaoli, P., 1996. CD8+ lymphocyte phenotype and cytokine production in long-term non-progressor and in progressor patients with HIV-1 infection. Clin. Exp. Immunol. 105 (2), 220–224.